A Three-Dimensional Culture Method to Expand Limbal Stem/Progenitor Cells.

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Public Summary:
The current standard method to culture human limbal stem/progenitor cells (LSCs) in vitro is to culture limbal epithelial cells directly on a layer of murine 3T3 feeder cells (standard method). The direct contact between human cells and murine feeder cells poses the potential risk of incomplete removal of feeder cells after culture and cross-contamination in clinical applications. We present here a novel three-dimensional (3D) sandwich method in which LSCs and feeder cells were separately cultured on opposite sides of a porous membrane. Limbal epithelial cells in the form of single-cell suspensions, cell clusters, and tissue explants were subjected to standard culture or to a 3D sandwich culture method. The 3D sandwich method consistently yielded LSCs derived from cell clusters and tissue explants. The expanded LSCs exhibited a small, compact, cuboidal stem-cell morphology and stem cell phenotypes comparable to those of LSCs derived from the standard culture method. Limbal epithelial cell clusters cultured with the sandwich method had a significantly higher proliferation rate than did those cultured with the standard method. The 3D sandwich method did not favor the propagation of single LSCs. In summary, the 3D sandwich method permits complete separation between cultured cells and feeder cells, while providing an even and maximal proximity between them. This alternative method permits culturing of LSCs without the risk of feeder cell contamination.

Scientific Abstract:
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